A.E. Wluka [†], C. Ding [‡], Y. Wang [†], G. Jones [‡], F.M. Cicuttini [†]. [†] *Monash Univ., Melbourne, Australia;* [‡] *Univ. of Tasmania, Hobart, Australia*

**Purpose:** Inflammation and vascular disease have recently been shown to play a role in the pathogenesis of osteoarthritis (OA). Low dose aspirin is commonly used in the prevention of cardiovascular disease. Its effects have been attributed to a variety of actions, including anti-inflammatory effects and effects on platelet function (both anti-thrombotic and anti-inflammatory) and lipids. However whether it affects human joints has not been studied. The aim of this study was to examine whether the use of low dose aspirin affects change in knee cartilage volume over 2 years.
**Methods:** 117 people with symptomatic knee OA underwent magnetic resonance imaging of the knee at baseline and 2 years later. Medial and lateral tibial cartilage volumes were measured using validated methods. Annual absolute change and annual percentage change in cartilage volume were calculated. Information about regular low dose aspirin use was collected at baseline, 6, 12 and 24 months. Participants who reported taking regular low dose aspirin (<= 150 mg per day) at more than 1 time point were defined as being aspirin users.
**Results:** Twenty six participants reported taking aspirin at more than one visit, with 91 not taking aspirin. At baseline, the only significant difference between the 2 groups was that those taking aspirin were older than those who did not ($p = 0.03$).
In those taking aspirin, annual change in medial tibial cartilage volume and annual percentage change in cartilage volume was approximately half that seen in those not taking aspirin (-50 vs. -102 mm3 and -2.5% vs. -5.5%, respectively, P=0.04 for both). These differences became more significant after adjusting for age, gender, body mass index, initial cartilage volume and severity of radiographic change in the medial compartment. The annual change in medial tibial cartilage volume was -40 mm3 (95% confidence interval (CI) -83, 1.3) in aspirin users vs. -105 mm3 (95% CI -127, -82) in non-aspirin users ($P = 0.009$ for difference). The annual percentage change in medial tibial cartilage volume was -2.0% (95% CI -4.6, 0.53) in aspirin users vs. -5.6% (95% CI -6.9, -4.0) in non-aspirin users (P=0.02 for difference). There were no significant differences observed in change in lateral tibial cartilage volume.
**Conclusion:** This study showed that in people with knee OA, the use of low dose aspirin was associated with reduced medial tibial cartilage loss over 2 years. This requires confirmation in a randomised controlled trial. If this hypothesis were proven, aspirin may provide a cost effective disease modifying therapy for OA as it is a cheap medication that is already in common use and known to be well tolerated.

## 462
## INTERACTIONS BETWEEN S100A8 AND DDR2 MECHANISMS IN CARTILAGE DEGRADATION IN OSTEOARTHRITIS

A. van Hummel [†,‡], L. Xu [§], C. Little [‡], T. Vogl [‖], J. Roth [‖], H. Zreiqat [†], Y. Li [§]. [†] *Tissue Engineering and Biomaterials Unit, Faculty of Engineering, The Univ. of Sydney, Sydney, Australia;* [‡] *Raymond Purves Bone and Joint Res. Lab., The Kolling Inst. of Med. Res., Univ. of Sydney, Royal North Shore Hosp., Sydney, Australia;* [§] *Dept. of Dev.al Biology, Harvard Sch. of Dental Med., Boston, MA, USA;* [‖] *Inst. of Immunology, Univ. of Muenster, Muenster, Germany*

**Purpose:** The surface bound tyrosine kinase receptor Ddr2 has been reported to play a role in early osteoarthritis (OA) pathogenesis. Increased levels of HtrA1 and degradation of the chondrocyte pericellular matrix expose collagen II fibrils, which activate Ddr2 upon binding, and lead to increased expression and activation of MMP-13. Pathology is delayed in surgically induced OA in *Ddr2*$^{+/-}$ mice, and Ddr2, HtrA1 and MMP-13 are all increased in a spontaneous model of OA in *Col11a1*$^{+/-}$ mice from 6 months of age. S100A8 and S100A9 have also been implicated in the induction of chondrocyte *MMP-13*, and are well known to be up-regulated at both the mRNA and protein level in inflammatory mouse models of arthritis. In contrast to inflammatory models, in a surgically-induced post-traumatic OA, chondrocyte S100A8 and S100A9 mRNA are up-regulated but they are not detected in cartilage at the protein level. The aim of this study was to determine if there is a link between the Ddr2 pathway and S100A8 and/or S100A9 in the pathogenesis of OA cartilage pathology by examining S100A8 and A9 expression in the *Coll11a1*$^{+/-}$ model where Ddr2 and HtrA1 are present, and by determining the effect of S100A8 and S100A9 on *Ddr2* and *HtrA1* expression in mouse cartilage.
**Methods:** S100A8 and S100A9 expression was examined in the knee cartilage of *Col11a1*$^{+/-}$ mice and wild-type (WT) littermates at 3 and 9 months of age using immunohistochemistry ($n = 3$ of both genotypes at both times; archival sections from mice with antigen-induced arthritis (AIA) used as a positive control).
Mouse femoral head cartilage explants were dissected from 15 WT mice between the ages of 6-8 weeks old, homogenised, and the explanted cartilage distributed evenly amongst wells, and cultured in serum-free media ± 0.1μM murine S100A8 or S100A9 for 24 hours (n=2 wells/treatment). Gene expression of *Ddr2, HtrA1* was determined by quantitative RT-PCR and normalised to *Gapdh* expression.
**Results:** S100A9 protein was not detected in the cartilage of 3 or 9 month old mice of either genotype although strong positive staining was observed in bone marrow of all mice, as well as cartilage from AIA animals. In addition to marrow and AIA cartilage samples, S100A8 protein was weakly detected in some chondrocytes/pericellular matrix in non-calcified cartilage in 1 of the 3 WT and *Coll11a1*$^{+/-}$ mice at 3 months of age. In contrast S100A8 was localized to chondrocytes/pericellular matrix of non-calcified cartilage and meniscal cells in all 9 month old animals, with no difference observed between genotypes. In mouse femoral cartilage explant cultures, gene expression of *Ddr2* was increased by S100A8, but decreased by S100A9, while *HtrA1* was decreased by both S100A8 and S100A9.
**Conclusions:** Whilst S100A8 staining was observed in non-calcified cartilage of some 3 month old and all 9 month old mice, there was no differential regulation between genotypes, suggesting that whilst an increase in S100A8 in cartilage may be an age-related change, it is not associated with the accelerated cartilage degradation seen in the *Col11a1*$^{+/-}$ model. In contrast, no S100A9 staining was observed in the cartilage of any mice from either genotype or age, and therefore may not be associated either with OA- or age-related cartilage changes. Gene expression data from femoral head cartilage cultures, however, suggests that S100A8 but not S100A9 can increase *Ddr2* mRNA, providing a link between these degradative cartilage pathways. However the down-regulation of *HtrA1* mRNA by the S100 proteins suggests that an alternative mechanism for exposure of the Ddr2 ligand would be required. Taken together, this data suggests that whilst S100A8 is not associated with cartilage degradation in *Col11a1*$^{+/-}$, it may pre-dispose the cartilage to degradation via up-regulation of *Ddr2*.

## 463
## PPARDELTA AS A NOVEL TARGET FOR OSTEOARTHRITIS THERAPY

A. Ratneswaran, E. LeBlanc, S. Ohora, M. Pest, F. Beier. *Western Univ., London, ON, Canada*

**Introduction:** Osteoarthritis (OA) is a degenerative disorder associated with the breakdown of articular cartilage. The mechanisms responsible for this condition are not well understood and therefore no treatments exist to halt or delay the progression of OA. Recent findings from our laboratory indicate that activation of the transcription factor PPARdelta induces the expression of enzymes involved in proteoglycan breakdown and can lead to cartilage degeneration in OA, prompting us to speculate whether inhibition of PPARdelta, can protect from cartilage breakdown in OA.
**Purpose:** To evaluate the role of PPARdelta in Osteoarthritis through all encompassing in-vitro, ex-vivo and in-vivo models of disease.
**Methods:** To test this, human chondrocytes and mouse femoral head cartilage explants were treated with pharmacological agonist (GW501516) and antagonists of PPARdelta (GSK3787/0660) to evaluate changes in gene expression (qPCR), and histology (Safranin-O, immunohistochemistry) consistent with OA, and to determine if recovery was possible.
Our in-vivo approach uses the Cre-Lox system to inactivate PPARdelta specifically in the cartilage of using a surgical model of OA. Mutant and control mice aged 20 weeks are being compared 8 weeks after a destabilization of medial meniscus (DMM) surgery, based on the principle that changes in biomechanical load drive cartilage degeneration. In order to assess the progression of OA between groups, histopathological scoring (OARSI) and immunohistochemistry for known markers of OA, (MMP 13, cartilage matrix breakdown products) are being analyzed. Serum analyses for extracellular matrix markers of cartilage breakdown are being conducted. To investigate changes in joint loading during OA, mutant and control mice are being compared through gait analyses using the CatWalk system that measures load on

individual limbs, stride length, stride pattern and velocity of movement, all of which can be affected by OA.

**Results:** Our data indicate upregulation in gene expression of catabolic enzymes involved in OA progression, and cartilage degeneration (MMP 2, 3, ADAMTS 4, 5) after agonist treatment, as well as pathophysiological markers of OA such as Aggrecan and Collagen II fragments in histological analyses.

**Conclusions:** Our study provides a comprehensive understanding of this gene's role in OA from both molecular and functional levels. These studies will establish whether inhibition of RRARdelta function is a valuable approach to treat OA.

## 464

## BASELINE VARUS THRUST AND 3-YEAR INCREASE IN DENUDED SUBCHONDRAL BONE IN PERSONS WITH OR AT HIGHER RISK FOR KNEE OSTEOARTHRITIS (OA)

A.H. Chang [†], J.S. Chmiel [†], O. Almagor [†], F. Eckstein [‡], K. Hayes [†], K.C. Moisio [†], M. Nevitt [§], K. Kwoh [‖], M. Hochberg [¶], C. Eaton [#], J. Bathon [††], R. Jackson [‡‡], D. Dunlop [†], L. Sharma [†]. [†] *Northwestern Univ., Chicago, IL, USA;* [‡] *Paracelsus Med. Univ., Salzburg, Austria;* [§] *Univ. of California, San Francisco, San Francisco, CA, USA;* [‖] *Univ. of Pittsburgh, Pittsburgh, PA, USA;* [¶] *Univ. of Maryland, Baltimore, MD, USA;* [#] *Brown Univ., Providence, RI, USA;* [††] *Columbia Univ., New York, NY, USA;* [‡‡] *Ohio State Univ., Columbus, OH, USA*

**Purpose:** Repetitive loading can damage articular cartilage and potentially expose the subchondral bone plate over time. Advances in knee MRI acquisition and cartilage and bone morphology measurement enable quantification of the area of subchondral bone void of articular cartilage (denuded bone area) in cartilage plates and specific subregions. It is unclear whether varus thrust, a mechanical risk factor for radiographic knee OA progression, relates to subsequent increase in the area of denuded bone. We hypothesized that baseline varus thrust presence is associated with a greater baseline-to-3-year increase in denuded bone in medial tibial and weightbearing femoral plates as well as in the central and external subregions within these plates.

**Methods:** The OAI is a prospective longitudinal study of persons with or at higher risk for knee OA aged between 45-79 years. Quantitative measures of tibiofemoral percent area of denuded bone were obtained from sagittal DESSwe MR images at the 12- and 48-month visits by expert readers who were blinded to acquisition order. Following a protocol and blinded to the MRI data, trained examiners assessed varus thrust presence as participants walked on a 10-meter walkway. Standing knee alignment was measured during physical examination with a long-arm goniometer using a standardized protocol. To examine the relationship between baseline varus thrust presence and change of area of denuded bone 3 years later, we used generalized linear models (multiple regression models) with GEE, with a continuous outcome variable for change in percent area of denuded bone over 3 years and covariates of age, sex, BMI, and knee alignment at baseline.

**Results:** 956 persons [57% women, mean age 60.5 years (8.9, SD), BMI 27.8 kg/m$^2$ (4.6, SD)] contributing 965 knees were included in the analysis. 266 (28%) knees had baseline varus thrust; 30% of participants were obese, 12% overweight, and 58% normal weight. The mean knee alignment was -0.15 degrees (3.70, SD). As shown in Table 1, having a baseline varus thrust was associated with a greater increase in percent denuded bone in the medial tibial and weightbearing femoral plates, especially in the central subregions, at 3-year follow-up. These relationships remained statistically significant after adjusting for age, sex, BMI, and knee alignment. Obese BMI, overweight BMI, and varus alignment were also associated with subsequent increase in subchondral plate bone exposure in most regions of interest (Table 2).

**Conclusions:** Varus thrust during walking was associated with a significantly greater, albeit modest, increase in percent denuded bone in the medial tibiofemoral joint, especially in the central subregion of medial weightbearing femoral plate. Overweight, obesity, and varus malalignment were also associated with greater increase in denuded bone.

**Table 1**
Comparison of knees with varus thrust vs. knees without varus thrust at baseline. Estimated baseline-to-3-year increase (A) in %area of subchondral bone denuded of cartilage due to varus thrust: Results based on generalized linear regression models with CEE, (n=965 knees, 956 persons)

| Additional baseline variables in model: | Medial tibial region [mean difference (95% CI) in Δ in %area of denuded bone, for varus thmst vs. non-vams thrust knees] | | | Medial weightbearing femoral region [mean difference (95% CI) in Δ in %area of denuded bone, for vams thrust vs. non-varus thrust knees] | | |
|---|---|---|---|---|---|---|
| | Entire region | Central subregion | External subregion | Entire region | Central subregion | External subregion |
| None | **0.70* (0.10,130)** | **1.09* (0.18,1.99)** | 1,71 (-0,09,3.50) | **1.29* (0.39,2.20)** | **2.16* (0.55, 3.76)** | 1.04 (-0.24, 2.32) |
| Adjusted for age, sex, and BMI | **0.61* (0.03,1.19)** | **0.98* (0.10,1.85)** | 1.43 (-0.28,3.14) | **1.12* (0.24,2.00)** | **1.82* (0.28,3.36)** | 0.74 (-0.48, 1.96) |
| Adjusted for age, sex, BMI, and knee varus alignment | **0.58* (0.01,1.16)** | **0.91* (0.04,1.77)** | 1.39 (-0.33,3.11) | **1.07* (0.20,1.94)** | **1.73* (0.20,3.26)** | 0.68 (-0.52, 1.87) |

*The outcome is a continuous variable for change in percent area of denuded bone over 3 years; 95% CI excluding 0 is statistically significant
- Age is a continuous variable (unit: year)
- BM1 is categorized as normal (<25 kg/m$^2$)(reference category), overweight (25-29.9), or obese (≥30)
- Knee varus alignment is a continuous variable (unit: degree)

**Table 2**
Association of baseline varus thrust, age, sex, BMI, and varus alignment on estimated baseline-to-3-year increase (Δ) in %denuded area of bone, for each ofthe final multivariate models of Table 1. (n=965 knees, 956 persons)

| Baseline variable: | Medial tibial region [mean difference (95% CI) in Δ in %area of denuded bone, for each variable in the model] | | | Medial weightbearing femoral region [ROB difference (95% CI) in Δ in %area of denuded bone, foi each variable in the model] | | |
|---|---|---|---|---|---|---|
| | Entire region | Central subrcgion | External subregion | Entire region | Central subregion | External subregion |
| Varus thrust present (vs. no varus thrust) | **0.58* (0.01,1.16)** | **0.91* (0.04,1.77)** | 1.39 (-0.33, 3.11) | **1.07* (0.20,1.94)** | **1.73* (0.20, 3.26)** | 0.68 (-0.52, 1.87) |
| Age (per 1 year) | 0.01 (-0.01, 0.04) | 0.03 (-0.003, 0.05) | 0.07 (-0.02, 0.16) | 0.02 (-0.01. 0.06) | 0.04 (-0.02, 0.10) | 0.04 (-0.01, 0.09) |
| Female (vs. male) | 0.28 (-0.18, 0.75) | 0.50 (.0.17, 1.18) | 0.33 (-1.07,1.73) | 0.25 (41.45, 0.94) | 0.37 (-0.86,1.60) | -0.17 (,1.13,0.82) |
| BMI obese (vs. normal) | **1.28* (0.69,1.88)** | **1.57* (0.74, 2.40)** | **3.29* (1.48,5.09)** | **2.15* (1 30, 3.01)** | **4.1.3* (2.59, 5.67)** | **3.05* (1.76, 4.33)** |
| BMI overweight (V5 normal) | 0.35 (-0.08, 0.79) | **0.59* (0.00,1.18)** | 1.02 (-0.36. 2.39) | **0.95* (0.37,1.54)** | **1.65* (0.72, 2.57)** | **1.39* (0.53, 2.26)** |
| Knee varus alignment (per 1 degree varus) | **0.08* (0.01,0.16)** | **0.19* (0.07,0.32)** | 0.12 (-0.10,0.34) | **0.15* (0.03,0.27)** | **0.27* (0.08, 0.45)** | **0.18* (0.01, 0.34)** |

*The outcome is a continuous variable for change in percent area of denuded bone over 3 years; 95% CI excluding 0 is statistically significant